THE EFFECT OF SEDIMENTATION ON BOVINE SPERM CELLS

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ON BOVINE SPERM CELLS

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CHAPTER I

INTRODUCTION

The separation of male determining and female determining spermatozoa has been sought for several years and is receiving research attention.

Many methods of separation of male determining and female determining spermatozoa have been tried with varied and always limited success. The method showing the greatest promise and receiving much research attention in the last two decades involved separation of spermatozoa by gravitational means. The fastest way to do this is by centrifuging the semen in a buffering solution. Sedimentation of a population of cells in appropriate media is believed to allow the X-bearing spermatozoa to settle down at a faster rate than the Y-bearing spermatozoa, thus partially separating the sperm into two populations. In principle, it is believed that the X-bearing spermatozoa are morphologically different from the Y-bearing spermatozoa, the former being larger and/or heavier than the latter.

Considerable work has been conducted in the separation of a single bull ejaculate into two different populations by sedimentation. In nearly all studies bovine and porcine spermatozoa have been used. Most researchers have utilized insemination trials rather than studying the multiple characteristics of the separated sperm populations to determine if separation was successful.

Limited studies have shown that morphological differences do exist between the two populations of separated cells but these characteristics cannot be attributed solely to X- and Y-bearing spermatozoa. Rather, there is the suggestion that the cells sediment to some degree according to the age of the cells. This study is devoted to the study of the motility and morphology of the two populations separated by sedimentation. It is hoped that a study of the motility characteristics and dimensions of the nucleus and acrosome will aid in the development of a method to alter the sex ratio.

CHAPTER II

LITERATURE REVIEW

Morphology of the Mammalian Sperm Cell

Morphologically the mammalian sperm cell consists of a head (nucleus) and a tail. The latter is composed of the neck, the mid piece, the main piece and the end piece. The entire cell is covered by a cell membrane.

The Head

Blom (1961) studied the ultra-structure of bull sperm and described the head as a tennis racket-like structure (dimensions 8 x 4 x 0.5 μ) composed mainly of a dense nucleus (0.2 - 0.3 μ thick) with a somewhat thicker base and a sharp pointed apex which formed a front bridge. The basal half of the nucleus was covered by a rather thin but relatively electron dense postnuclear cap. The apical half of the nucleus was covered by a somewhat thicker (0.1 μ) and less electron dense cap, the acrosome.

Blom and Birch-Andersen (1965) found that the nucleus portion of bull sperm was covered by two different systems of membranes. The most peripherally located was the cell membrane which enveloped the entire sperm cell. Between the cell membrane and the nuclear membrane a more complicated membrane system was found, the posterior part of which was the post nuclear cap and the anterior part was the acrosome cap. The

latter was given different names by different researchers, i.e., acrosomic cap and the anterior cap by Rahlmann (1961); acrosome by Nicander and Bane (1962 and 1963); acrosome cap by Bedford (1963 and 1964) and by Blom and Birch-Andersen (1965); head cap, acrosome-head cap complex by Hadek (1963 and 1964); and head cap by Saacke and Almquist (1964).

Saacke and Almquist (1964) reported that the nucleus is covered anteriorly by a three-layered head cap and posteriorly by a loose thin postnuclear cap. They also reported that a sequence of structural alterations in the head cap provided evidence for the appearance of the equatorial segment. The anterior portion of the head cap swelled and deteriorated leaving the posterior portion intact as a band about the center of the nucleus. That band represented the equatorial segment.

The Tail

Blom and Birch-Andersen (1965) described the part of the tail that attached to the head of sperm as the capitulum of the tail which was composed of two implantation plates together with the proximal centriole. They also noticed that the tail capitulum fitted tightly to the implantation groove (a concave recess of the head).

Bishop and Austin (1957) described the tail as a long (40-50 μ) flagellum differentiated into four regions: the neck, the mid-piece, the main piece and the end piece. The neck being the short anterior extremity; the mid-piece characterized by a double mitchondrial helix surrounding the axial core, the latter composed of twenty discrete fibres running from the neck throughout the tail; the main piece being the longest part of the tail provided most of the propellant capability. They also noticed that the tail ended in a short (3-10 μ) distal end

piece which was not surrounded by a helical sheath.

The Cell Membrane

Bedford (1963) demonstrated in rabbit sperm, a close apposition of the cell membrane and the membrane of the apical body at its lateral and anterior edges. This was also confirmed in bull sperm by Blom and Birch-Andersen (1965). The latter authors reported that the cell membrane enveloped the entire sperm head and tail. Blom and Birch-Andersen (1965) also noticed that the cell membrane was often quite loosely attached to the mitochondrial sheath of the middle piece, a phenomenon emphasized by Saacke and Almquist (1964).

Size Characteristics of Sperm Cells

Some of the methods used in attempting to separate X and Y sperm cells assume that there is a difference in either the size or mass of these cells. Several references will be presented to characterize the size or mass of the cells, the variation that is typical among animals and within ejaculates and how separation procedures affect sperm characteristics.

Specific Gravity

Lindahl and Kihlstrom (1952) used Umbradit salts of methylglucamine as the suspending medium and found bull spermatozoa to have a range in specific gravity of 1.241 to 1.335 (7.5%). Lindahl and Thunqvist (1965) used Ficoll (polysaccharide of glucose) and obtained values of 1.21 to 1.33 for bull spermatozoa. Beatty (1964) used dialyzed colloidal silica and obtained a lower specific gravity, 1.132, for rabbit spermatozoa.

Yamane (1920) also reported a lower value, 1.0975, for stallion spermatozoa.

Andersen (1946) used the fall-drop technique for determining specific gravity and obtained similar values for the specific gravity of bull seminal plasma and whole semen (1.0267 - 1.0392). Lavon et al. (1966) used phthalate esters and obtained values ranging from 1.0376 to 1.092 of specific gravity of bull spermatozoa, nearly similar to what Andersen (1946) observed. Variability in specific gravity among ejaculates apparently varied as much as 5%.

Bendict et al. (1967) separated a normal population of spermatozoa by zonal isopycnic centrifigation into two density classes; a low density class and a high density class. The low density sperm had a buoyant density of 1.16 - 1.19 g/ml and the high density sperm 1.25 - 1.32 g/ml, or, about a 7% difference.

These values obtained by different researchers demonstrated a wide difference in specific gravity for bull sperm from 1.0267 to 1.335 (30%).

Mass

Lenchtenberger (1956) used interference microscopy for determining dry mass of bull spermatozoan and obtained a dry mass of 7.1×10^{-12} g for nuclei with normal DNA content. Muller et al. (1959) used microradiography and obtained a value of 7.87×10^{-12} g for bull sperm head but when using interference microscopy he obtained a value of 8.94×10^{-12} g.

Bahr and Zeither (1964) used electron microscopy (photographic transmission of an electron micrograph, taken under appropriate conditions is proportional to the mass per area of the object producing

that transmission) and obtained the following masses for the different bull sperm parts (X 10^{-12}g): head 13.35 ± 0.70, tail 4.85 ± 0.3, mid-piece 2.31 ± 0.15, whole sperm 18.20 ± 0.65. Carlson and Glendhill (1966) found a similar value of 13.2 x 10^{-12}g, for the mass of the sperm cell head using microradiography.

The difference in dry mass found in the various studies might be due to the different techniques, ejaculates differences or breed differences.

Dimensions of the Bull Sperm

Reported measurements of the head and other parts of bull spermatozoa varies considerably. Savage et al. (1927) found average head length of bull spermatozoa to be 8.5 μ with a coefficient of variation of 3.5%. Van Duijn (1960) reported mean values of 10.2 μ and 5.4 μ for head length and width of bovine spermatozoa, respectively,with coefficients of variations of 4.9% for head length and 6.5% for head width. Bretherton (1961) reported a range of 8.5 - 10.0 μ for head length and 4.5 μ for head width.

Bahr and Zeitler (1964) used electron microscopy (cells were photographed then the micrographs were enlarged to a magnification of 5,000 and then direct measuring was carried out) and obtained a value of 8.83 μ ± 0.25 for the head length, 53.4 μ ± 1.20 for the tail and 11.0 μ ± 0.3 for the middle piece.

Mukherjee and Singh (1965) used an ocular screw micrometer (X15) and an oil-immersion objective (final magnification x 1,319) for the measurements of the different parts of bull sperm (using stained slides) of three different breeds. They obtained significantly different

($P < .01$) values of 9.630 μ, 9,280 μ and 8,9392 μ ± 0.0571 μ for the head length of the three breeds and 5.0053 μ, 5,2195 μ and 5.2974 μ ± 0.0571 μ for the sperm width of the same breeds. Later in 1966 they observed a significant effect of season on both head length and head area.

Wells, et al. (1973) used a binocular microscope fitted with a micrometer eye piece and an oil immersion objective lens (100x) to study the size characteristics of sperm cells of 11 bulls. They obtained a mean value of 8.30 ± 0.43 μ for the head length and a mean value of 4.53 μ ± 0.33 μ for the head width. Differences among bulls for both characteristics were significant ($P < .01$). Head length varied up to 20% and head width varied up to 37% within ejaculates.

The various measurements utilized indicate wide ranges in sperm size an/or mass.

Trials to Separate Sperm Cells Into X- and Y-Chromosome-Bearing Cells by Sedimentation

Attempts to separate the spermatozoa population of a single mammalian ejaculate on the supposed difference in size of the sex cells have been made since the pioneering experiments of Lush (1925), followed successively by many other researchers. Such attempts have commonly employed sedimentation of sperm by gravity or by centrifugation. Separation in these experiments depended mainly on the size and weight of sperm cells. The media used had a primary characteristic of furnishing a suitable density gradient for separation considering as much as possible the preservation of motility and reasonable survival of spermatozoa.

Lush (1925) using rabbits, was the first to investigate the possi-

bility of sex control by using centrifuged spermatozoa. He diluted semen with Ringer's solution which resulted in a distinct separation of spermatozoa after centrifugation. Using the top and bottom fractions of the centrifuged diluted semen in insemination trials, he did not achieve a deviation in sex ratio in the progeny produced.

Lindahl and Kihlstrom (1952) attempted to separate bull spermatozoa according to size by the use of counter-current centrifugation. The principle of which was sketched by Lindahl (1948). They centrifuged sperm cells in a density gradient medium thus permitting sperm cells of greater density to sediment. They showed that changes in specific density or volume occurred during the maturation of sperm cells. They also showed that aged sperm cells had a higher specific density than younger cells.

Lindahl (1956) used the fastest sedimenting bull spermatozoa, separated by counter-streaming centrifugations (1,100 r.p.m.) for insemination trials and unexpectedly, the first 11 calves from 23 inseminations were males. Fertility was low in his experiments and he proposed that female determining cells were more sensitive to mechanical damage by the counter-streaming centrifugation.

In 1958, Lindahl also used spermatozoa of bulls separated by counter-streaming centrifugation (1,100 and 1,000 r.p.m.) and from 142 inseminations, 63 calves were produced with no significant deviation of the sex ratio. He stated that low fertility was a problem associated with centrifuged cells. He also found that relatively high velocity centrifugation reduced fertility more than relatively low centrifugation velocity.

Bhattacharya (1958 and 1962) described a method for separating

rabbit semen into two fractions with which he achieved a significant degree of predetermination of sex. He obtained 75% of females in litters of rabbits produced from insemination with fractions sedimenting at a faster rate and 80% males in litters of rabbits produced from using fractions sedimented at a slower rate. His experiment was based on a small sample. He suggested that the separation depended on the tendency of the female producing X-sperm to sediment more rapidly than the male producing Y-sperm when the sperm were rendered immotile by cooling and then allowed to sink under the effect of gravity for 12 hours through a column of viscous egg yolk medium. Bhattacharya et al. (1966) sedimented cells on 2 kinds of media, namely, egg yolk/glycerine and milk/glycerine. In insemination trails, they found that the sex distribution of progeny was not significantly different.

Andersen and Rottensen (1962) used sedimented rabbit spermatozoa in insemination trials and obtained a preponderance of females in the progeny produced, regardless of the sedimentation rate.

Beatty (1964) used a dialyzed colloidal silica on rabbit spermatozoa and found it to be an excellent density gradient medium for motile spermatozoa but fertility was adversely affected. Sperm cell morphological characteristics were not studied.

Beatty (1969) confirmed that differential density of X- and Y-bearing-spermatozoa was not the operative factor for their separation. His research was conducted on rabbits using dextran-based density gradients for separation. He used fast sedimenting spermatozoa and of 90 offspring produced 44 were males and 46 were females, i.e., no deviation from normal sex ratio.

Schilling (1966) used a medium consisting of skim milk, variable

concentrations of salt solutions and egg yolk and of sp. gr. 1.037 and 1.044. This medium permitted shortening the time of sedimentation from 12 hours to 1 hour and the motility and survival of spermatozoa was decreased only by 10%. He reported that he had achieved 10-12 fractions of spermatozoa in this medium. Counting cells in different fractions he found a unimodal curve. Bhattacharya (1962), using a similar approach, obtained a bimodal curve. Schilling (1966) inseminated a number of cows with the lower fractions and out of 86 calvings, there were 69.8% female calves born. He believed that he had successfully isolated X- and Y-bearing-sperm and that his achievements supported the hypothesis that X-bearing-spermatozoa are larger and heavier than Y-bearing-spermatozoa.

Bedford and Bibeau (1967) used the same medium and apparatus used by Schilling (1966) on rabbit spermatozoa. They rendered the cells immotile by slowly cooling them to $2^{o}C$ in 2 hours. They found a bimodal sperm sedimentation pattern in contrast to the consistent unimodal sedimentation pattern observed by Schilling (1966). In two sets of their insemination trials (8 does in the first series and 6 does in the second) using the lower fractions no preponderance of females was achieved; however, a higher percentage of females also resulted from inseminations with the upper fractions. The sex ratios achieved in this study should be viewed with caution due to extremely limited numbers of animals in the insemination studies.

Knaack (1973) inseminated 2,358 cows with sedimented bull spermatozoa and found 66% females as a consequence of insemination with faster sedimenting sperm and 67% males resulted from insemination with the slower sedimenting fractions.

Krazanowski (1970) separated bull spermatozoa by sedimentation at

2^{o} - 4^{o}C using three kinds of media made of skim milk and egg yolk. He inseminated 2,866 cows with sedimenting spermatozoa. He obtained a significant preponderance of females (372 males/423 females) from those inseminated with fast sedimenting fractions but no alteration of sex ratio when using slow sedimenting sperm. He suggested that the more rapid sedimentation rate of sperm with an X chromosome was cuased by their greater ability to agglomerate. Sperm characteristics were not reported, however, it was stated that conception rate was lower and abortion rate was higher with sedimented semen. This would indicate that some critical sperm characteristics were different for these cells.

Kiddy and Bahr (1968) studied the weight of the upper and lower fractions of bull spermatozoa with media composed of 55% fresh egg yolk and 45% of a 20% glycine solution. After sedimentation was achieved they found that the median dry mass of populations of sperm from the bottom fractions of the columns ranged from 3.0 to 6.8% heavier than the sperm from the top fractions.

O'Donnell and Symons (1969) centrifuged bull spermatozoa in various density gradients in a zonal rotary centrifuge rotor. They found that a significantly greater proportion of cells had lost the acrosome in heavier fractions as compared with lighter fractions. Since acrosome degradation and loss were known to be indicators of cell senescence, they concluded that centrifugation of bull spermatozoa on density gradients resulted in a distribution which reflected the age of the cells.

Schilling (1971) summarized his work on the sedimentation of spermatozoa and concluded that the sex ratio of calves born from inseminations with sedimented sperm in cattle was changed to as much as 70% male or female progeny indicating a concentration of X- and Y-chromosome-bearing

spermatozoa in bottom or top fractions, respectively.

Bahr (1971) summarized all the attempts to separate male and female spermatozoa in four decades, and concluded that the techniques for separation were inadequate and that the subject has not been pursued with sufficient vigor or at sufficient depth; secondly, the separation was not possible for reasons of principle. He stated that complexity and variability of spermatozoa precluded clear-cut separation of X- and Y-bearing cells. Three major types of variability which might appear possible were: (a) interspermatozoal variability, (b) variability among ejaculates from the same donor, and (c) interdonor variability.

Factors Affecting Sedimentation Rate

In addition to the variability described by Bahr (1971) other factors apparently affect the sedimentation process. Schilling (1971) stated that two components of the sedimentation medium, egg yolk and milk powder have variable composition mainly due to variations in water and fat content or to manufacturing variations. These variations can affect sedimentation and they prevent exact standardization of the medium. Previously Kampschmidt et al. (1951) found that the major factors causing the variation in the amount of settling of egg yolk and semen plasma were: (a) amount of mixing, in which settling varied with the degree of mixing; in thoroughly mixed, little or no settling occured; (b) ratio of egg yolk to citrate; here with smaller ratios of egg yolk to citrate, settling was at a faster rate; (c) concentration of electrolytes in which case settling of egg yolk solids could be prevented by replacing all or part of the buffer solution with non-electrolytes.

Schilling (1971) found that for most bulls, sedimentation separation

of sperm occured in media with specific gravities between 1.035 and 1.042 (20°C) and vescosities 5.0 to 7.0 cP. He also observed that several bulls were unsuitable for sperm separation due to the variable size of shape and divergent specific gravity of their sperm. Lindahl and Kihlstrom (1952) reported that specific gravity of bull spermatozoa increased during maturation and aging of sperm. The size and number of particles in the medium were also shown to have an effect but no detailed investigations on their role was published.

Most studies to separate bull spermatozoa into X- and Y-bearing populations relied heavily on limited insemination trials to determine their success. They did not thoroughly characterize the morphological characteristics of these two populations. Some of them have reported that sedimentation has harmful effects on sperm cells. Consequently, this study was conducted to evaluate the following sperm characteristics for the two fractions separated:

(1) percent motile

(2) rate of motility

(3) percent abnormal

(4) acrosome characteristics

(5) cell dimensions

CHAPTER III

MATERIAL AND METHOD

Semen Collection and Handling

Semen for this study was collected biweekly from four bulls housed in separate pens with adjacent runs at the University dairy farm. Collection began on November 1, 1974 and concluded on November 25, 1974. Collection of ejaculates was generally achieved by using an artificial vagina. Electro-ejaculation was utilized once on one of the bulls who was reluctant to cooperate in the artificial vagina collection procedure.

Before semen was collected, the underline of each bull was thoroughly washed and any long hair around the preputial opening was clipped thus avoiding to some extent the possibility of gross contamination.

After each collection, the semen was maintained in a water bath ($37^{o}C$) while initial ejaculate characteristics were determined.

Experimental Design and Statistical Analysis

Semen was collected from 4 bulls on 5 collection days. For each ejaculate, one million sperm cells in 1 ml of seminal plasma, cooled to $2^{o}C$, were layered onto sedimentation columns containing 19 ml of the prepared cooled ($2^{o}C$) medium secured in burettes 48 cm long with 1 cm inner diameter each. Then the sperm were allowed to separate for 1 hour. The upper 2 ml fractions and the lower 2 ml fractions were removed and characterized for this study. Differences among bulls, replications,

and fractions in percent motile cells, rate of movement, acrosome characteristics and cell morphology were tested by analysis of variance utilizing a split-plot design (Steel and Torrie, 1970). Bulls were considered as blocks with replicates (dates) as whole unit treatment and fractions as sub-unit treatment. Replicate X fraction, bull X fraction and bull X fraction X replicate interactions were included in the analysis. If replicate X fraction was significant, comparisons were made between replicate-fraction subclasses. The whole unit error term used was bull X replicate (two-way interaction error term) and the subunit error term was a pooled term including bull X fraction and bull X replicate X fraction. These analyses utilized the Statistical Analyses System (SAS) computer program developed by Barr and Goodnight (1972).

In the analysis of variance conducted, treatment involved the upper and lower fractions only, initial ejaculate was excluded.

Determination of Initial Ejaculate Characteristics

The following ejaculate characteristics were determined prior to sedimentation.

1. Semen volume was measured by direct reading to the nearest 0.1 ml from the graduated collection tubes used.

2. Sperm motility: The percentage of progressively motile spermatozoa for each ejaculate was estimated usually, in increments of 5%, by microscopic examination of a small drop of semen diluted with warmed ($35^{o}C$) sodium citrate (2.9%).

3. Rate of motility was determined concomitantly from the motility slides. Rates were estimated from zero to 4, in increments of .5, with

zero denoting no progressive motile sperm and rates of 2, 3, and 4 denoting gradation to maximum progressive motility.

4. Sperm concentration: This was determined with a spectrophotometer ("Spectromic 20"), previously calibrated with haemocytometer counts, by combining .05 ml semen and 7.95 ml of 2.9% sodium citrate solution. Percent light transmission values were converted to number of sperm per ml by reference to prepared tables.

5. Percent live sperm: This was determined for each ejaculate using the nigrosine-eosin live-dead differential stain (Hancock, 1952) prepared by combining 5 qt. water soluble cosin Y in 300 ml of a 10% solution of nigrosin. One drop of semen for each sample was mixed in a staining vial with four drops of live-dead stain and left to stand in a warm stage ($37^{o}C$) for 15 seconds. After the fifteen seconds, one drop of the suspension was smeared on a slide on the warm stage and allowed to air dry. A total of 200 spermatozoa for each slide was made if possible. On a few slides, only 100 cells were counted. The dead cells stained red while the live cells were unstained. Partially stained cells were considered dead.

6. Acrosomal characteristics: These were determined for each ejaculate using the Wells-Awa (1968) acrosome stain. This stain was prepared by combining one volume of a 1.0% solution of eosin B (88% total dye content), two volumes of a 1.0% solution of fast green FCF (90% total dye content), and 1.7 volumes of ethyl alcohol. For preparing sperm smears, semen was first diluted 1:10 with 2.9% sodium citrate, then four drops of stain and one drop of semen was combined in a staining vial. This combination was then mixed, let stand for three minutes, and then a small drop of the stain-sperm mixture was placed on a warmed

clean slide and smeared in a thin layer. The smeared slide was then air dried, examined for readable distribution of cells and a clean cover slip was applied with diaphan. Slides were examined with high, dry magnification (430X). The condition of the acrosome was determined by examining a total of 200 cells, when possible, if not, a minimum of 100 cells was considered. Cells were first classified as either normal or abnormal and the acrosome was then classified as aged or non-aged within cell type.

A non-aged sperm cell was one which showed a uniformly smooth acrosome that was closely adherent to the nucleus and was free from any evidence of swelling or disintegration. Aged cells were identified according to the acrosomal deterioration they showed; acrosomal deterioration has been shown to be correlated with senscence of spermatozoa (Saacke and Marshall, 1968). Morphological changes which were considered indicative of some stage of aging were:

(a) swollen or thickened acrosome, characterized by partial or complete swelling of the outer membrane of the acrosome cap and thus an enlargement in acrosomal size,

(b) elevated acrosome, characterized by a swollen and elevated acrosome that is becoming detached from the nucleus,

(c) roughened acrosome cap, characterized by a partial or entire roughened acrosome surface,

(d) enlarged equatorial segment, characterized by a half moon shape of the equatorial segment becoming exaggerated as the acrosome becomes loosened from the cell head,

(e) disintegrating acrosome cap, characterized by the rupture of the outer and inner acrosomal membrane showing the later stage of aging,

(f) capless cell or detached acrosome where the acrosome is completely missing and is considered as the terminal stage of aging. In this stage the upper portion of the nucleus stains light pink instead of the typical green when the acrosome is present,

7. Abnormal sperm count was determined from the stained slides used for acrosomal characteristics by combing the abnormal non-aged and the abnormal aged categories.

Cells considered abnormal contained at least one of the following characteristics: pinhead, gianthead, pyriform head, twin head; hooked, coiled, broken, bent, filiform or twin tail; immature (with protoplasmic drops on the mid piece) and cells with double mid pieces.

The average dimensions of the cells in the initial, upper and lower fractions were determined by measuring 25 cells on the acrosome stain slides. Measurements were made with the oil immersion objective (100X) of a phase contrast microscope fitted with a micrometer eyepiece in one of the ocular tubes. This device was capable of measuring .01 micron.

Measurements were made of the following parts of the sperm head: head length (excluding the top part of the acrosome), head width (excluding the acrosome width on both sides of the cell), total cell width (including the acrosome on both sides of the cell), acrosome width (determined by subtracting the nucleus width from the total cell width and then dividing by 2), and the thickness (top part of the acrosome) as a deviation from the proximal part of the nucleus.

Sedimentation Procedure

Preparation of the Medium

The medium, apparatus and procedure for sedimentation in this study

were adopted from Kampschmidt et al. (1951), Schilling (1966), Bedford and Bibeau (1967), Krazanowski (1970), and Lavon et al. (1971).

The medium was routinely prepared the night before semen collection, as follows:

1. 7.6 g powdered skim milk were dissolved in 64 ml distilled water, heated to 90-92°C for 10 minutes and filtered.

2. 13 ml of sodium citrate solution (2.9%), 13 ml of glucose solution (3.75%), 18.5 ml fresh egg yolk, 1 ml warmed gelatine (10%) and 1.0 mg of an antibiotic containing equal amounts (by weight) of crystalline penicillin (1585 i.u./mg) and streptomycin sulphate dissolved in 2 ml distilled water were then gently mixed with the milk solution and stored in a refrigerator before use the following morning.

Preparation of Spermatozoa for Sedimentation

All sedimentation work was carried out in the University dairy farm cold room.

The amount of semen to be used for sedimentation for each bull was one ml adjusted by centrifugation (using a rotor centrifuge) to contain one million cells. The samples were then cooled slowly to 2°C in two hours to render the sperm immotile.

Apparatus Used and Sedimentation Technique

The materials used for sedimentation consisted of:

(a) Four burettes each of which was 48 cm long with an outer diameter of 1.2 cm, inner diameter of 1.0 cm and with an outlet 0.1 cm in diameter with a stopcock 6 cm from the outlet opening.

(b) Four 2,000 ml cylinders with the following dimensions each:

44 cm long, 7.0 cm outer diameter and 6.5 cm inner diameter.

(c) Four burette stands with four clips.

Nineteen ml of the prepared medium was placed in each of the four burettes. The burettes, held by the clips on the stands, were immersed in ice water in the large cylinders. Then the media was cooled to 2°C by adding more ice to the ice water. Cooling of the media occurred concomitantly with cooling of the sperm suspensions. Then after two hours, the sperm suspensions (one ml each) were carefully layered on top of the media and were then left for one hour to accomplish sedimentation.

After sedimentation was allowed for one hour, 2 ml were gently and cautiously taken from the top fractions with a pipette. Another two ml sample was taken from the lower fractions by gently opening the stopcock at the bottom of each burette. Two samples, one from the upper fractions and the other from the lower fractions were secured for each bull for each ejaculate. The samples, put in small tubes, were then labelled and immersed in beakers containing cold water ready for recovery of the sperm cells.

Recovery of the Sedimented Spermatozoa

For recovery of spermatozoa the following was done:

(a) 0.5 ml from each fraction were placed in labelled centrifuge tubes and centrifuged for 2 minutes at approximately 1700Xg.

(b) the supernatant was then decanted from each tube bearing a pellet in the bottom as undisturbed as possible.

(c) the pellet was then resuspended in 0.5ml of cold sodium citrate (2.9%), centrifuged for two minutes and supernatant decanted.

(d) the pellet was then resuspended in 0.5 ml of warmer citrate

solution (2.9%) centrifuged for two minutes and supernatant was decanted.

(e) the above step was repeated and then the sperm were resuspended in 0.5 ml sodium citrate.

(f) at that point samples were ready for motility estimates and staining for live-dead slides and acrosome slides.

Determination of Sedimented Sperm Characteristics

After the spermatozoa from the upper and lower fractions were recovered, the sperm characteristics were characterized as described for initial ejaculates.

CHAPTER IV

RESULTS AND DISCUSSION

All 20 samples of semen collected from the four bulls used for this study had normal semen characteristics. After sedimentation in the egg yolk-milk medium, spermatozoa were present in the 2 fractions collected. The lower fraction was usually discernible and distinct for all ejaculates. The upper fractions did not show visually distinct layering in the sedimentation process. Schilling (1966, 1971) used a similar medium and reported the separation into 10-12 fractions in bull sperm. This present study did not attempt to determine the total number of fractions achieved by the sedimentation procedure. Preliminary investigations established that motile cells were present throughout the sedimentation column after one hour of sedimentation.

The pH of the medium used was 6.8, viscosity of the medium was 7.18 cP and the relative density (sp. gr.) was 1.043 at the top of the medium column and 1.050 at the bottom of the column two hours after preparation of the columns and cooling to $2^{\circ}C$.

Motility Characteristics

Table I summarizes the percent motile cells prior to and after sedimentation and Table II presents the analysis of variance for this characteristic for sedimented sperm. There was a higher mean percentage ($P < .05$) of motile cells in the lower fraction (48.3% vs. 35.4%).

TABLE I

PERCENT MOTILE SPERM PRIOR TO AND AFTER SEDIMENTATION

Bull No.	Initial Ejaculate	Upper Fraction	Lower Fraction
		Percent	
1	77.00	44.60	53.00
2	75.00	19.00	53.00
3	76.00	36.00	21.00
4	82.00	42.00	66.00
Mean	77.50	35.40	48.25

S.E. for bull = $\pm$ 6.60
S.E. for fraction = $\pm$ 4.31

TABLE II

ANALYSIS OF VARIANCE FOR PERCENT MOTILE SPERM

Source	df	SS	MS	F Value	Level of Significance
Bull	3	4083.68	1361.23	3.11	
Date	4	2396.65	599.16	1.37	
Bull x Date	12	5245.95	437.16		
Treatment	1	1651.23	1651.23	4.45	$P < .05$
Date x Treatment	4	796.15	199.04	0.54	
Bull x Treatment	3	3417.68	1139.23		
Bull x Date x Treatment	12	2144.45	178.70		

Table I also shows that this same pattern was present for all bulls. The percent motile cells for both fractions was significantly reduced from that of the initial ejaculates. It would appear that separation has occured on the basis of motility. However, it should be recalled that the sedimentation was carried out at 2°C which immobilizes the sperm cells (Bedford and Bibeau, 1967; Krazanowski, 1970; Lavon et al. 1971). Moreover, the velocity of bull sperm was determined to be 30 ± 2.6 μ/sec in an egg yolk-citrate diluent (Van Duijn, 1961) and the length of the sedimentation column used in this study was 19 cm so it was not possible for the sperm to move that distance in one hour. Therefore, some other factor must be considered as the prime contributor causing sedimentation. This could be explained by the assumption that spermatozoa do not sediment separately but in agglomeration (Krazanowski 1970). In this study head agglutination was noticed suggesting that agglomeration did occur. It would also appear that the sedimentation medium has a deleterious effect on sperm cells since the motility in both fractions was sharply reduced from initial evaluation. Differences among bulls and dates or any of the possible interactions were not significant for this sperm characteristic.

Tables III and IV reveal the same effects on the rate of sperm motility with the lower fractions having a higher ($P < .01$) rate, 3.25, than the upper fraction, 2.70, a 20% advantage. This is not in agreement with the 20% slower velocity of the "heavy" sperm (lower fraction) reported by Schilling and Schmid (1967). This could be due to media or technique differences. The medium apparently had an adverse effect on this characteristic as both fractions scored lower than the initial ratings. There was no significant effect of bull, date or possible

TABLE III

RATE OF MOTILITY OF SPERM PRIOR TO AND AFTER SEDIMENTATION

Bull No.	Initial Ejaculate	Upper Fraction (a)	Lower Fraction
1	3.90	3.10	3.50
2	3.90	2.10	3.30
3	4.00	2.70	2.60
4	3.90	2.90	3.60
Mean	3.93	2.70	3.25

(a) Rate is from 0 to 4
S.E. of the mean for bull = $\pm$ 0.23
S.E. of the mean for fraction = $\pm$ 0.26

TABLE IV

ANALYSIS OF VARIANCE FOR RATE OF MOTILITY

Source	df	SS	MS	F Value	Level of Significance
Bull	3	3.63	1.21	2.27	
Date	4	3.98	0.99	1.87	
Bull x Date	12	6.38	0.53		
Treatment	1	3.03	3.03	12.52	P < .01
Date X Treatment	4	1.85	0.46	1.91	
Bull x Treatment	3	2.23	0.74		
Bull x Date x Treatment	12	1.40	0.12		

interactions on rate of motility.

Table V gives a summary of total abnormal cells prior to and after sedimentation, indicating that the lower fraction consistently has a higher percentage ($P < .01$) of abnormal cells than the upper fraction. No significant interaction was found among bulls or replicates (Table VI) but a significant interaction ($P < .05$) between treatment and bull was found. Examination of Table VII shows that on some replicates the cell response was different than on other replicates. It is known that each ejaculate collected will have some percentage of abnormal cells and may respond somewhat differently to routines imposed.

An attempt was made to utilize the live-dead stain described by Hancock (1952) for staining the cells in the sedimented fractions. This was considered unsuccessful due to some contradictory observations that some samples which visually exhibited a high percent of motile cells showed very low, or no live cells on the stained slides.

Acrosome Characteristics

The mean percentages and analysis of normal, non-aged cells are presented in Tables VIII and IX. The percent normal cells showing no aging of the acrosome decreased for both fractions from the initial ejaculate characteristics. The upper fraction was superior ($P = .0001$), 80.03%, to the lower fraction, 75.55%. Differences among bulls and replications and any possible interactions were not significant. These data agree with the observations of O'Donnell and Symons (1969) that heavier fractions of centrifuged sperm showed greater alteration of the acrosome.

Percent normal cells with aged acrosomes were also determined and

TABLE V

TOTAL ABNORMAL SPERM PRIOR TO AND AFTER SEDIMENTATION

Bull No.	Initial Ejaculate	Upper Fraction	Lower Fraction
	----------Percent----------		
1	6.40	6.80	9.30
2	4.50	6.10	8.10
3	6.60	7.98	9.30
4	4.80	5.80	9.60
Mean	5.58	6.67	9.08

S.E. of mean for bull = $\pm$ 0.91
S.E. of mean for fraction = $\pm$ 0.39

TABLE VI

ANALYSIS OF VARIANCE FOR PERCENT ABNORMAL SPERM

Source	df	SS	MS	F Value	Level of Significance
Bull	3	12.47	4.16	0.50	
Date	4	84.14	21.03	2.55	
Bull x Date	12	99.09	8.26		
Treatment	1	57.84	57.84	21.06	$P < 0.01$
Date x Treatment	4	59.57	14.89	3.88	$P < 0.05$
Bull x Treatment	3	8.24	2.75		
Bull x Date x Treatment	12	49.40	4.12		

TABLE VII

TOTAL ABNORMAL SPERM AFTER SEDIMENTATION

Replicate	Upper Fraction	Lower Fraction
	Percent	Percent
1	7.88	12.75
2	4.50	7.25
3	8.25	6.13
4	5.73	9.88
5	7.00	9.38
Mean	6.67	9.08

S.E. of the mean for replicate = $\pm$ 1.02
S.E. of the mean for fraction = $\pm$ 0.39

TABLE VIII

PERCENT NORMAL SPERM WITH NON-AGED ACROSOMES
PRIOR TO AND AFTER SEDIMENTATION

Bull No.	Initial Ejaculate	Upper Fraction	Lower Fraction
	----------Percent----------		
1	87.40	80.90	74.60
2	90.60	83.10	80.00
3	90.10	80.60	75.20
4	87.20	75.50	72.40
Mean	88.83	80.03	75.55

S.E. of the mean for bull = $\pm$ 1.97
S.E. of the mean for fraction = $\pm$ 0.53

TABLE IX

ANALYSIS OF VARIANCE FOR PERCENT NORMAL SPERM WITH NON-AGED ACROSOMES

Source	df	SS	MS	F Value	Level of Significance
Bull	3	288.97	96.32	2.49	
Date	4	94.23	23.56	0.61	
Bull x Date	12	464.38	38.69		
Treatment	1	200.26	200.26	35.04	P = 0.0001
Date x Treatment	4	38.90	9.73	1.70	
Bull x Treatment	3	19.92	6.64		
Bull x Date x Treatment	12	65.80	5.48		

the means and analysis of this characteristic are presented in Tables X and XI. The mean percentages between fractions were similar, suggesting that the significant reduction in the percent normal cells with non-aged acrosomes (discussed above) in the lower fraction was accompanied by an increase in one of the abnormal categories. This is verified by examination of the mean percentages of abnormal cells with aged acrosomes in Table XIV. Analysis revealed that the 4.3% observed in the lower fraction was significantly higher ($P < .01$ Table XV) than the 2.45% in the upper fraction.

There was no difference between the upper and lower fractions in mean percentage of abnormal non-aged cells (Tables XII and XIII). Likewise, there were no significant interactions between bull or replicate for any of the preceding acrosome characteristics.

These significant differences revealed in percentage of total abnormal cells, percentage normal cells with non-aged acrosomes and percentage abnormal cells with aged acrosomes between the upper and lower fractions are probably due to the medium acting as a density gradient and separating cells to some degree on the basis of aging or senescence of the cells.

The mean percentages and analysis of total percent aged acrosomes (the combination of normal aged and abnormal aged) are presented in Tables XVI and XVII. The difference of 4% between the upper and lower fractions (inferiority of the lower fraction by 20%) was highly significant ($P < .01$). The percent aged acrosomes in both fractions was higher than the initial indicating that the medium had a detrimental effect on acrosomes. There were no significant differences among bulls or replicates, nor were there any significant interactions.

TABLE X

PERCENT NORMAL SPERM WITH AGED ACROSOMES
PRIOR TO AND AFTER SEDIMENTATION

Bull No.	Initial Ejaculate	Upper Fraction	Lower Fraction
	----------Percent----------		
1	6.20	12.30	16.10
2	4.90	10.80	11.90
3	3.30	11.42	15.50
4	8.00	18.70	18.00
Mean	5.60	13.30	15.36

S.E. of the mean for fraction = $\pm$ 0.78
S.E. of the mean for bull = $\pm$ 1.84

TABLE XI

ANALYSIS OF VARIANCE FOR PERCENT NORMAL SPERM WITH AGED ACROSOMES

Source	df	SS	MS	F Value	Level of Significance
Bull	3	258.14	86.05	2.54	
Date	4	46.53	11.63	0.34	
Bull x Date	12	407.03	33.92		
Treatment	1	42.85	42.67	3.49	$P < .1$
Date x Treatment	4	90.68	22.67	1.84	
Bull x Treatment	3	39.12	13.04		
Bull x Date x Treatment	12	145.68	12.14		

TABLE XII

PERCENT ABNORMAL SPERM WITH NON-AGED ACROSOMES
PRIOR TO AND AFTER SEDIMENTATION

Bull No.	Initial Ejaculate	Upper Fraction	Lower Fraction
	----------Percent----------		
1	4.50	4.10	4.30
2	3.60	3.80	4.70
3	4.90	5.78	4.90
4	4.00	3.20	5.20
Mean	4.25	4.22	4.76

S.E. of the mean for bull = $\pm$ 0.78
S.E. of the mean for fraction = $\pm$ 0.45

TABLE XIII

ANALYSIS OF VARIANCE FOR PERCENT ABNORMAL SPERM WITH NON-AGED ACROSOMES

Source	df	SS	MS	F Value	Level of Significance
Bull	3	9.48	3.16	0.53	
Date	4	47.06	11.77	1.96	
Bull x Date	12	72.16	6.01		
Treatment	1	3.08	3.08	0.77	P < .61
Date x Treatment	4	33.98	8.49	2.11	
Bull x Treatment	3	10.98	3.66		
Bull x Date x Treatment	12	49.36	4.11		

TABLE XIV

PERCENT ABNORMAL SPERM WITH AGED ACROSOMES
PRIOR TO AND AFTER SEDIMENTATION

Bull No.	Initial Ejaculate	Upper Fraction	Lower Fraction
	----------Percent----------		
1	1.90	2.70	5.00
2	0.90	2.30	3.40
3	1.70	2.20	4.40
4	0.80	2.60	4.40
Mean	1.33	2.45	4.30

S.E. of the mean for bull = $\pm$ 0.44
S.E. of the mean for fraction = $\pm$ 0.35

TABLE XV

ANALYSIS OF VARIANCE FOR PERCENT ABNORMAL SPERM WITH AGED ACROSOMES

Source	df	SS	MS	F Value	Level of Significance
Bull	3	5.23	1.74	0.89	
Date	4	25.81	6.45	3.31	P < .05
Bull x Date	12	23.34	1.94		
Treatment	1	34.22	34.22	14.09	P < .01
Date x Treatment	4	8.84	2.21	0.91	
Bull x Treatment	3	2.23	0.74		
Bull x Date x Treatment	12	34.21	2.85		

TABLE XVI

PERCENT AGED ACROSOMES PRIOR TO AND AFTER SEDIMENTATION

Bull No.	Initial Ejaculate	Upper Fraction	Lower Fraction
		Percent	
1	9.1	15.0	21.1
2	5.8	13.1	15.8
3	5.0	13.8	19.9
4	8.8	21.3	22.4
Mean	6.7	15.8	19.8

S.E. of the mean for bull = $\pm$ 1.99
S.E. of the mean for fraction = $\pm$ 0.80

TABLE XVII

ANALYSIS OF VARIANCE FOR PERCENT AGED ACROSOMES

Source	df	SS	MS	F Value	Level of Significance
Bull	3	302.94	100.98	2.55	
Date	4	97.22	24.30	0.61	
Bull x Date	12	475.82	39.65		
Treatment	1	149.77	149.77	11.65	P < .01
Date x Treatment	4	76.13	19.03	1.48	
Bull x Treatment	3	50.79	16.93		
Bull x Date x Treatment	12	142.09	11.84		

The increase in percent aged acrosomes indicates that the medium hastened the aging process and then the aged cells tended to sediment more rapidly toward the lower fractions of the columns. This is also in agreement with O'Donnell and Symons (1969) who had shown that density gradient centrifugation resulted in a distribution of bull spermatozoa which reflected the aging condition of the cells with a progressive acrosomal degeneration apparent with increasing sedimentation rate. This could also possibly have some relationship to the lowered fertility rate and increased abortion rate reported by Lindahl (1956 and 1958) and Krozanowski (1970) when using sedimented populations of sperm cells.

Cell Measurements

Table XVIII summarizes the mean sperm head dimensions of the spermatozoa of the four bulls prior to sedimentation. Each value of head parameter is a mean of 125 measurements (25 cell measurements per bull for each ejaculate).

Table XIX shows the effect of sedimentation on sperm head dimensions. This table is a guideline for the other tables which will follow. The head length, head width, nucleus length and nucleus width for the two fractions are smaller than the same parameters for the initial indicating that the sedimentation medium affected both fractions. The mean head length and head width for both fractions indicate that cells in the lower fractions were larger than those in the upper fractions ($P < .01$ for head width) which is in agreement with what Schilling (1971) found (9.46μ and 9.24μ for head length, and 5.25μ and 4.96μ for head width for lower and upper fractions, respectively) although he found no differences in the relative sizes of the acrosomes.

TABLE XVIII

SPERM HEAD DIMENSIONS PRIOR TO SEDIMENTATION

Bull No.	Sperm Head Characteristics					
	Total Head Length	Total Head Width	Nucleus Length	Nucleus Width	Acrosome Width (side)	Acrosome Thickness (Apex)
1	9.34	5.09	8.62	4.36	0.36	0.72
2	9.42	5.18	8.72	4.42	0.38	0.70
3	9.39	5.07	8.71	4.35	0.36	0.69
4	9.51	5.33	8.80	4.52	0.40	0.71
Mean	9.42	5.17	8.71	4.41	0.38	0.71

Measurements in microns

TABLE XIX

EFFECT OF SEDIMENTATION ON SPERM HEAD DIMENSIONS

Parameter	Upper Fraction	Lower Fraction	Difference
			--Percent--
Total Head Length (μ)	9.27	9.33	1
Total Head Width (μ)	5.03	5.15	2.4[a]
Nucleus Length (μ)	8.43	8.34	1[b]
Nucleus Width (μ)	4.26	4.21	1.2
Acrosome Width (side) (μ)	0.38	0.47	23.7[c]
Acrosome Thickness (top) (μ)	0.84	0.99	17.9[d]

$a_P < .01$ $b_P < .05$ $c_P < .05$ $d_P = .0001$

Means and analysis of total sperm head length and width (including acrosome) are presented in Tables XIX, XX, XXI, and XXII. The difference in the sperm head length (.06μ) between the two fractions approaches significance ($P < .1$). The head width was 2.4% larger in the lower fraction, a highly significant difference ($P < .01$). In both length and width characteristics there was a highly significant date effect ($P < .01$ and $P < .001$ for length and width respectively). This may be reflecting differences in the general maturity of each ejaculate of cells. The number of days between ejaculates varied from three to five which likely would have some influences on sperm size. There was also a significant bull difference in sperm width ($P < .05$) which agrees with Wells et al.(1973) who observed significant animal differences in cell dimensions.

The lower fractions being larger in size than the upper fraction agrees with the findings of Krajnc (1964) and Schilling et al.(1967).

Means and analysis of nucleus length and width (exclusive of the acrosome) are shown in Tables XIX, XXIII and XXIV. The difference of 1% between the two fractions in nucleus length was significant ($P < .05$). However, nucleus width was not significantly different. The date effect ($P < .001$) was significant in the analysis of both parameters as were bull differences for nucleus width ($P < .05$). It should be pointed out that the nucleus dimensions were actually somewhat smaller in the lower fraction. This is contradictory to the contention of some that the X-bearing sperm cells (nuclei) are larger in size. This does not preclude the possibility that these cells may be denser (Van Duijn, 1968).

Examination of Tables XIX, XXV and XXVI shows that there were significant differences in acrosome dimensions between the upper and lower

TABLE XX

SPERM HEAD DIMENSIONS AFTER SEDIMENTATION

Bull No.	Sperm Head Length		Sperm Head Width	
	Upper Fraction	Lower Fraction	Upper Fraction	Lower Fraction
1	9.26	9.26	4.97	5.24
2	9.29	9.38	5.09	5.10
3	9.34	9.32	4.95	4.99
4	9.18	9.35	5.11	5.25
Mean	9.27	9.33	5.03	5.15

S.E. of a mean for bull for head length = $\pm$ 0.11
S.E. of a mean for treatment for head length = $\pm$ 0.02
S.E. for bull (head width) = $\pm$ 0.21
S.E. for treatment (head width) = $\pm$ 0.04
Measurements in microns

TABLE XXI

ANALYSIS OF VARIANCE FOR SPERM HEAD LENGTH

Source	df	SS	MS	F Value	Level of Significance
Bull	3	1.21	0.40	2.11	
Date	4	5.94	1.48	7.78	P < .01
Bull x Date	12	2.29	0.19		
Treatment	1	0.89	0.89	3.05	P < .1
Date x Treatment	4	0.94	0.24	0.80	
Bull x Treatment	3	1.38	0.46		
Bull x Date x Treatment	12	3.02	0.25		

TABLE XXII

ANALYSIS OF VARIANCE FOR SPERM HEAD WIDTH

Source	df	SS	MS	F Value	Level of Significance
Bull	3	5.72	1.91	4.51	P < .05
Date	4	22.83	5.71	13.52	P < .001
Bull x Date	12	5.07	0.42		
Treatment	1	3.36	3.36	8.85	P < .01
Date x Treatment	4	6.96	1.74	4.59	P < .01
Bull x Treatment	3	2.71	0.90		
Bull x Date x Treatment	12	2.98	0.25		

TABLE XXIII

ANALYSIS OF VARIANCE FOR NUCLEUS LENGTH OF SPERM

Source	df	SS	MS	F Value	Level of Significance
Bull	3	1.48	0.49	5.77	P < .05
Date	4	13.54	3.39	39.56	P = .0001
Bull x Date	12	1.03	0.09		
Treatment	1	1.99	1.99	5.54	P < .05
Date x Treatment	4	1.00	0.25	0.69	
Bull x Treatment	3	1.01	0.34		
Bull x Date x Treatment	12	4.36	0.36		

TABLE XXIV

ANALYSIS OF VARIANCE FOR SPERM NUCLEUS WIDTH

Source	df	SS	MS	F Value	Level of Significance
Bull	3	2.44	0.81	3.26	
Date	4	15.90	3.89	15.62	P < .001
Bull x Date	12	2.99	0.25		
Treatment	1	0.62	0.62	3.07	P < .1
Date x Treatment	4	0.32	0.32	1.61	
Bull x Treatment	3	0.07	0.07		
Bull x Date x Treatment	12	0.23	0.23		

TABLE XXV

ANALYSIS OF VARIANCE FOR ACROSOME WIDTH (SIDE) OF SPERM

Source	df	SS	MS	F Value	Level of Significance
Bull	3	0.63	0.21	5.99	
Date	4	3.79	0.95	24.30	
Bull x Date	12	0.47	0.04		
Treatment	1	1.72	1.71	10.21	P < .05
Date x Treatment	4	2.01	0.50	6.92	
Bull x Treatment	3	0.50	0.17		
Bull x Date x Treatment	12	0.59	0.05		

TABLE XXVI

ANALYSIS OF VARIANCE FOR ACROSOME THICKNESS (TOP) OF SPERM

Source	df	SS	MS	F Value	Level of Significance
Bull	3	0.07	0.02	0.21	
Date	4	1.79	0.45	4.11	P < .05
Bull x Date	12	1.31	0.11		
Treatment	1	5.55	5.55	57.87	P = 0.0001
Date x Treatment	4	0.27	0.07	0.70	
Bull x Treatment	3	0.26	0.09		
Bull x Date x Treatment	12	1.18	0.09		

fractions. The lower fraction showed greater alterations ($P < .05$) in acrosome width (.47μ) than did the upper fraction (.38μ). Similarly, the thickness of the acrosome at the apex of the cell was significantly greater ($P = .0001$) in the lower fraction, .99μ, than in the upper fraction, .84μ. Difference in bull and dates were not significant for acrosome width but date was significant for acrosome thickness which may be explained the same as far as other date interactions. The sperm cell size data and acrosome data combined would add additional support to the conclusion that the cells sediment to some degree according to their total size which is influenced greatly by the acrosome.

CHAPTER V

SUMMARY AND CONCLUSIONS

Semen was collected from four bulls on five different days to study the effect of sedimentation on spermatozoa characteristics. Egg yolk-milk medium was used for sedimenting spermatozoa into the two different populations.

The technique and medium used worked satisfactorily. Sedimentation into two morphologically different sperm populations was achieved. The live-dead stain was unsuccessful so percent live was not quantified.

The characteristics of spermatozoa in the upper and lower fractions were studied and analysis of the data revealed superiority ($P < .05$) in percent motile cells and rate of motility of the cells of the lower fraction. An increase in percentage of abnormal cells in the lower fraction was found ($P < .01$).

A higher percentage ($P = .0001$) of normal cells with non-aged acrosomes was found in the upper fraction. There was no significant difference between the fractions in percent normal cells with aged acrosomes or abnormal cells with non-aged acrosomes. The lower fraction had a higher percentage ($P < .01$) of abnormal cells with aged acrosomes and a higher total percentage of aged acrosomes ($P < .01$). Twenty-five sperm cells from each fraction for all ejaculates were measured to determine how sedimentation affected size characteristics. Nucleus length in the upper fraction was greater ($P < .05$) than that of the lower fraction

while nucleus width was similar. The total size of the cells (inclusive of acrosome dimensions) was larger in the lower fractions with sperm width being significantly greater ($P < .01$).

Analysis of acrosome dimensions for the upper and lower fractions showed that acrosomes in the lower fraction averaged significantly wider ($P < .05$) at the side of the cell and at the apex of the cell ($P = .0001$) than those in the upper fraction.

The characteristics of the sperm cells in the two fractions compared in this study indicate that fractionation did occur. However, there is no evidence to support the supposed size difference in the sex cells that is sought in sex ratio control studies. It would appear that the primary factor influencing sedimentation in this study was the increase in acrosome size rather than a difference in nucleus size.

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